Search for Clinical Evidence of an Exoerythrocytic Stage in Mice Exposed to *Plasmodium gallinaceum* Infection.* (28967)

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Using *P. gallinaceum* sporozoites as infecting organisms, *A. aegypti* as vector, 6- to 9-day old White Rock chicks as susceptible hosts, and 3- to 4-week old male albino mice as non-susceptible hosts, I(1) found practically immediate inactivation of the organism when deposited by the mosquito in the skin of the mouse, whereas survival was very good for at least an hour in the skin of the chick. The present investigation was designed to determine whether the sporozoites may have become established in exoerythrocytic form in the spleen of the mouse though their survival in the skin could not be demonstrated.

Methods. Forty mice with shaved bellies, held securely for 5 minutes on top of globes containing 20 to 25 infected mosquitoes, received an average of 4.9 potentially-infecting bites. As controls, 40 chicks with the down plucked from the breast were handled in the same way, and received an average of 4.6 bites. Then at a later time the spleens of all animals were removed and introduced intraperitoneally into clean chicks, employing a modification of the technic of Coulston, Cantrell and Huff(2). The whole spleen was transferred from bitten chick to clean chick, but only about one-fourth of the organ was transferred from mouse to chick. After the third day, blood smears were made from each of the bitten animals daily, including the day of splenectomy, to determine whether the infection had reached the stage of blood patency before the operation. Ten chicks and 10 mice were splenectomized on the fourth day after the infecting bites, and 10 of each on the 5th, 6th and 7th days. The trials were scattered through many weeks, with only a few animals being exposed to any one batch of mosquitoes, to insure that the infectiousness of the vectors would be truly representative of that through which propagation of the organism is continuously accomplished in this laboratory. All chicks that did not develop infection within 21 days of receiving implants from either chicks or mice, were subjected to infecting bites to derive substantiating evidence, in the case of resultant infections, of failure to receive infecting material in the implants.

Results. The data displayed in Table I reveal that infection occurred in all 20 of the chicks that received spleens from chicks bitten 6 or 7 days previously, and that it occurred also in 14 of the 20 receiving spleens from chicks that had been bitten 4 or 5 days previously. It is also shown in the Table that none of the chicks receiving spleens of bitten mice became infected no matter when the implants were made. There is also evidence, in the last right-hand column in the Table, that the chicks apparently uninfected by the implants had truly not become infected, since they developed infection when bitten by infected mosquitoes shortly thereafter.

Discussion. The fact that all 10 chicks became infected when implanted with splenic material from chicks bitten by infected mosquitoes 7 days earlier, does not necessarily indicate that the infections resulted from forms released from exoerythrocytic sites in the organ. In 6 of the 10 donor chicks involved there was already an erythrocytic blood-stream infection at time of the donation, and presumably there could have been erythrocyte infections in the other 4 as well, even though much more prolonged search of the slides than is usual in routine procedures in the laboratory had failed to reveal them. However, the likelihood that infections resulted from transfer of infected erythrocytes to chicks implanted only 6 days after infecting bites is much smaller, for only 1 of 10 of these chicks had a blood-stream infection at time of the implants, and it is only occasionally that we find blood-stream patency at

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TABLE I. Infectability of Spleens of Susceptible (Chick) and Non-susceptible (Mouse) Species Bitten by *A. aegypti* Mosquitoes Vectoring *P. gallinaceum*.

Donors		Days to implant	No. positive donor bloods at implant	No. positive recipient bloods after implant	No. negative recipient chicks infected by later bites
Chicks	10	7	6	10	
"	10	6	1	10	
"	10	5	0	7	3
"	10	4	0	7	3
Mice	10	7	0	0	10
"	10	6	0	0	10
"	10	5	0	0	10
"	10	4	0	0	10

6 days in our propagating routines with *P. gallinaceum*. It is extremely unlikely that the 7 infections resulting from 10 implantations from chicks 5 days after their infecting bites, and the similar number after only 4 days, could have been due to transfer of infected erythrocytes; we never find organisms in the blood-stream this early following the bites of so few mosquitoes as were used here. In the case of the splenic implants from mosquito-bitten mice, there is no evidence that they contained viable forms of any sort, since no infections resulted from their presence in clean chicks no matter how long after the bites the implants were made.

Summary. When *mice* were bitten by *A. aegypti* mosquitoes infected with *P. gallinaceum* sporozoites, and portions of their spleens were subsequently implanted intraperitoneally in clean chicks after intervals of 4 to 7 days, the latter did not develop infections in any instance. When similar implantations were made from bitten *chicks*, there resulted an impressive number of infections that were not easily ascribable to transfer of infected erythrocytes from the blood-stream. The evidence strongly suggests that the well-known exoerythrocytic cycle of *P. gallinaceum* in the chick, a species that is fully susceptible to infection with sporozoites of the organism, does not find a counterpart in the mouse, a species that is not demonstrably susceptible to such sporozoite infection.

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Estimation of Frequency of Occurrence of Galactosemia in the Population. (28968)

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Since the discovery of the biochemical defect in the hereditary disease of galactosemia as a deficiency in galactose-1-phosphate uridyl transferase(1), a number of procedures have been developed to identify the homozygous diseased individual(2). These can be applied with certainty to give chemical support to the clinical diagnosis.

In several family studies where parents, siblings and other relatives have been sampled it has become obvious that identification of the heterozygous carrier of the dis-

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